Interfacial Protein-Lipid Interactions I

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Revealing Conformational Substates of Lipidated N-Ras Protein by Pressure Modulation

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Signaling networks maintain their spatiality by localization of their protein constituents to distinct regions of the membrane, and the different Ras guanine nucleotide binding proteins are a paradigm example of this. Despite being highly homologous, they exhibit isoform specific diversity in generating explicit signal outputs governed by, but not limited to, their hyper variable region responsible for targeting them to particular membrane microdomains (1). In addition, Ras proteins are known to sample multiple conformations which exhibit varying affinities towards their interaction partners. To fully explore the conformational space exhibited by Ras, experimental identification of conformational substates and characterization of conformational equilibria are mandatory. We applied pressure modulation in combination with FT-IR spectroscopy to reveal equilibria between spectroscopically resolved, otherwise low lying, substates of the lipidated signaling protein N-Ras in its different nucleotide binding states and in the absence and presence of a model biomembrane. Not only the nucleotide binding, but also the presence of the membrane has a drastic effect on the conformational dynamics and selection of conformational substates of the protein, and a new substate appearing upon membrane binding could be uncovered. Population of this new substate is accompanied by structural reorientations of the G-domain involving α-helix-membrane interactions. These findings thus illustrate that the membrane controls signaling conformations by acting as an effective interaction partner which has consequences for the G-domain orientation of membrane-associated N-Ras which in turn is known to be critical for its effector and modulator interactions. Finally, these results provide first insights into the influence of pressure on Ras-controlled signaling events in organisms living under extreme environmental conditions as they are encountered in the deep sea.

Reference

1. Weise K, Kapoor S, ... Waldmann H, Winter R (2011) *J Am Chem Soc* 133:880-887.

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Importance of Aromatic Anchor Residue Identity and Location for the Tilt and Dynamics of Transmembrane Peptides

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Aromatic or polar amino acid residues often flank each side of a membrane-spanning alpha helix, whether in an integral membrane protein or a model peptide. The aromatic residues Trp, Tyr and to a lesser extent Phe tend to partition to the membrane-water interface and act as âœanchorsâ to stabilize the transmembrane orientation. The synthetic model peptide, GWALP23 (acetyl-GGALW^5(LA)$_6$LW^{19}LAGA-[ethanol]amide), has proven valuable for experimentation, with only one Trp âœanchorâ near each end of the transmembrane sequence. Indeed, with relatively minimal complications from the peptide dynamics, the average tilt of GWALP23 has been shown to vary systematically in lipid bilayer membranes of different thickness (see J. Biol. Chem. 285, 31723). We have employed ^{2}H-alanines and solid-state NMR spectroscopy to investigate the consequences of moving or replacing W5 or W19 in GWALP23 with selected Tyr, Phe or Trp residues at the same or nearby locations. We find that GWALP23 peptides having Y5, F5 or W5 exhibit essentially the same average tilt in bilayer membranes of DOPC, DMPC or DLPC; with somewhat increased dynamics for the F5 peptide. When âœdoubleâ anchors are present in Y^{4,5}GWALP23 or F^{4,5}GWALP23, the peptides appear less responsive to the bilayer thickness, as the dynamics become dramatically more extensive. Moving W19 to position 18, a 100° radial change, alters the direction of the helix tilt, as expected. We conclude that, in the absence of other functional groups, the aromatic residues determine the preferred orientations and dynamics of transmembrane peptides. Increased dynamics are observed when the ring hydrogen bonding is removed (Phe), or when two aromatic anchors are present on one side of the core transmembrane sequence.

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Response of GWALP Transmembrane Peptides to Titration of a Buried Lysine

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Designed α-helical peptides such as GWALP23 serve as useful models for probing the influence of polar amino acids within a core transmembrane helical sequence. We incorporated lysine as a guest residue into the membrane-spanning host peptides GWALP23 and closely related Y^{5}GWALP23 (acetyl-GGAL(W^5/Y^5)LALALAL^{12}AL^{14}ALALW^{19}LAGA-amide). Lysine was introduced at either position 12 or 14 of the host sequences, for which position 12 corresponds to the center. Solid-state NMR spectra of ^{2}H-Ala residues in peptides incorporated into oriented lipid bilayer samples reveal that L14K mutant peptides adopt well-defined orientations in DOPC, DMPC and DLPC. In each lipid membrane, the L14K substitution increases the helix tilt at neutral pH. The L12K substitution, on the other hand, reduces the ^{2}H NMR spectral quality at neutral pH, particularly in the thicker DOPC, suggesting a lack of distinct orientation, as the system struggles to insert a charged lysine into the thicker bilayers. As the positively charged K12 amino group is titrated to higher pH values, nevertheless, the ^{2}H NMR spectral quality improves in DOPC, and the K12 peptides adopt an average orientation nearly matching the one found for both host peptides GWALP23 and Y^{5}GWALP23 (with L12). In similar fashion, titration of K14, in any of the tested lipid bilayer membranes, results in a smaller helix tilt, again much closer to that observed for the L14 peptides without a polar guest residue. Steady-state fluorescence measurements using the Y^{5}GWALP23 series of peptides reveal spectral narrowing and modest blue shifts in Δ_{max} from the W19 reporter, when either K12 or K14 is rendered non-ionized, suggesting a somewhat more hydrophobic environment for the Trp indole ring when the guest Lys side chain is neutral.

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Characterization of Antimicrobial Peptides Relating to Shortened RWALP Model Peptides

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In the face of increasing bacterial drug resistance, small membrane-active peptides offer potentially attractive avenues to alternative antimicrobial agents. The goals of this project are to characterize the lipid interactions and analyze the antimicrobial efficacy of model peptides that are longer than the lactoferrin-derived, surface-acting LfB6 (RRWQWR-NH$_2$), yet shorter than the transmembrane RWALP23 (acetyl-GRALW(LA)$_6$LW-LARA-NH$_2$). New-generation RWALP peptides, of general sequence (ac-GR$_n$W$_m$(LA)$_j$LW$_m$R$_n$A-NH$_2$) were designed with varying total lengths (13-15) and numbers of Arg and Trp residues. ^{2}H-alanines were incorporated at several positions to serve as probes for recording solid-state NMR spectra from mechanically aligned samples of the peptides in bilayers of DLPC, DMPC or DOPC. Labeled RWALP13 (j=3, n=1, m=1) exhibits partial water solubility, no antimicrobial activity, and ^{2}H-NMR spectra characteristic of isotropic motion, even in the presence of lipid bilayers. Circular dichroism spectra of RWALP13 suggest partial α-helical character in water that is enhanced when lipids are present. The ^{2}H-NMR spectra indicate that the longer 14- or 15-residue peptides are aligned to varying degrees in the different lipid bilayer membranes. Antimicrobial assays reveal that peptides with four arginines have higher activity than those with only two arginines. Interestingly, within the 4-Arg category, RRWALP15 (j=3, n=2, m=1) shows higher activity (MIC of 6.25 μ1/4g/ml) against E. coli than does RRWWALP15 (j=2, n=2, m=2; MIC of 25 μ1/4g/ml). The ^{2}H-NMR spectra of RRWALP15 suggest significant alignment of the peptide with respect to lipid bilayer membranes. The combined antimicrobial and spectral features make RRWALP15 an especially good candidate for further analysis.

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Membrane Interactions of an Acylated and Non-Acylated Lactoferricin Peptide by Solid-State NMR and Fluorescence Spectroscopy and Molecular Dynamics Simulations

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LfB6 (RRWQWR-NH$_2$) is a tryptophan- and arginine-rich cationic antimicrobial peptide, derived from bovine lactoferrin, with broad spectrum activity that can be enhanced by N-terminal acylation (CH$_3$(CH$_2$)$_4$CO-RRWQWR-NH$_2$; C6-LfB6). The arginines promote selective interaction with negative